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An Experimental Study on the Influence of Cortisone on Pregnancy in the Albino Rat

Samuel Perelmuter
Loyola University Chicago

AN EXPERIMENTAL STUDY ON THE INFLUENCE OF CORTISONE ON PREGNANCY IN THE ALBINO RAT

by

Samuel Perelmuter

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LIFE

Samuel Perelmuter was born on February 13, 1919, in Ostrog, Poland. He was graduated from the Gymnasium in Ostrog, Poland, June, 1938, and completed his college undergraduate studies at the University of Munich, Germany in June, 1953.

The author began his graduate studies in the Department of Anatomy, Stritch School of Medicine, Loyola University, Chicago, in September, 1955.

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INTRODUCTION

During the past few years valuable and intensive work has been directed toward clarifying the fundamental mechanism of the action of cortisone in the normal and diseased organism. Although significant effects on the various tissues and organs have been demonstrated, the effects of the administration of cortisone during pregnancy have not been sufficiently investigated. The purpose of this investigation was to acquire more specific information on the effects of cortisone on the mother and fetus when administered prior to and during pregnancy in the albino rat. The existing similarity between the chemical structure of cortisone and that of certain of the sex hormones adds to the interest and significance of such a study.

It has not yet been demonstrated that the mode of action of cortisone can be attributed to any single circumscribed mechanism. The effects of cortisone probably also include undiscovered reactions on the chemical processes of organs, tissues, and cells of the organism. Not only has the variety and complexity of the effects of cortisone given much stimulus to investigative work in the field of experimental biology and medicine but the dosages administered have often produced such varied results that further study and research should prove both promising and rewarding. Furthermore, there is no unanimity of agreement with respect to the effects of cortisone on fertility or the course of pregnancy and parturition when administered prior to or during pregnancy. It

therefore seemed desirable to undertake an investigation with particular reference to the effects of cortisone on reproduction in the female.

REVIEW OF LITERATURE

In 1930 Swingle and Pfiffner prepared the first extract of the adrenal cortex capable of maintaining life in adrenalectomized animals. Since that time increasing attention has been directed to studies on extracts of the adrenal cortex. The word "cortisone" is a name introduced by Dr. E. C. Kendall of the Mayo Foundation in 1936 to identify a crystalline hormonal compound, originally called "Compound E", present in cortical extracts. On September 21, 1948, 100 mg. of cortisone were first injected into a patient with rheumatoid arthritis by Dr. Hench at the Mayo Clinic. The methods for synthesizing and producing cortisone have improved remarkably since that time. Cortisone is a 11 - dehydro - 17 hydroxycorticosterone.

Experimental evidence suggests that the administration of cortisone to animals during pregnancy may be harmful to the fetus. Courrier and Cologne (1951) gave pregnant rabbits 25 mg. of cortisone daily for four to seven days and later found the fetuses to be small, resorbed or macerated. The mortality rate of fetuses was investigated also by Leroy and Domm in the same year. The latter workers injected 500 gamma of cortisone subcutaneously into rat fetuses. Between the 11th and 16th days of gestation, all fetuses were killed, although normal size and survival were noted after injection between the 16th and 19th days. Domm and Leroy in 1955 also observed that cortisone causes modifications

in development and growth, particularly premature eruption of incisors in the rat. The effects of cortisone on rabbit pregnancy were discussed by De Costa and Abelman in 1952. They gave daily intramuscular doses of 5 to 20 mg., generally 15 mg. of cortisone during pregnancy, and disruption of gestation was noticed. Velardo (1957) studied the effect of ACTH on pregnancy and litter size in albino rats. Dosage was 2.0 to 4.0 mg. daily for 5 days prior to cohabitation. All ACTH treated groups produced stillbirths to the extent of 3 to 6 (16-40%) rats in each litter, while the controls had no abortions or stillbirths. According to Davis and Plotz (1954) injections of 3 mg. of cortisone daily for 16 to 19 days caused a high fetal mortality rate (47.7%). When the same dose was given for 6 to 9 days the mortality rate was not higher than 17.3%. Despite contradictory clinical reports experimental evidence collected by De Costa and Abelman (1952) and Katzenstein and Alston (1954) suggest that steroid therapy in the human during pregnancy does not interfere with fetal development or survival.

Ovarian and uterine weights were reported by Moore in 1953. He divided 127 rats into 22 groups, and injected cortisone subcutaneously in daily doses of 0.5 to 5.0 mg. for 7 to 14 days. The treated animals showed consistently heavier ovaries, while fresh weights of uteri did not vary perceptibly between the treated and untreated animals. Antopol (1950) found that the ovary and accessory reproductive organs of immature mice treated with 1.25 mg. of cortisone weighed less than normal. In contrast to the above, Elivaiss, Hanson, Rosenzweig, and McNiel (1954) reported marked increases in ovarian and uterine

weights. Their experiments were carried out with 24 pairs of pair-fed female rats given 1 mg. of cortisone, daily subcutaneously for 2 or 4 weeks starting at 23 days of age. These authors suggested that the increased weight of ovaries appeared to be due to a greater follicular development.

Since substances injected into the pregnant animal must pass the so-called "placental barrier" in order to reach the fetus a statement concerning our present knowledge of this structure seems appropriate. There are conflicting reports in the literature with regard to the structure of the placenta in the rat. According to Mossman (1937) the rat's placenta is hemochorial and appears to become hemoendothelial in late pregnancy. The rat placenta would then be the closest approach to an intermingling of fetal and maternal blood. Wislocki, Deane and Dempsey (1945) inclined to the view that the "labyrinthine trophoblast" in late pregnancy does not disappear and reported a basement membrane around each of the rats' fetal capillaries. According to Bridgman (1948) the cytoplasm of the labyrinthine trophoblast persists until term. Wislocki and Dempsey (1955) utilizing the electron microscope reinvestigated the structure of the placenta and concluded that the placental barrier is not hemoendothelial, but hemochorial. If true, this would indicate that the human and rat have the same type of placenta which may imply a similar passage of metabolites. On the other hand, the overlapping cellular character of the trophoblast of the rat placenta, shown by the electron microscope studies, may have far different transfer properties than the single layer synctium of the human placenta.

MATERIALS AND METHODS

Albino rats of a standard strain of Sprague-Dawley were used in our experiments. All animals were given Purina "Fox-chow" pellets and tap water ad libitum. Cortisone, Cortone Acetate, generously supplied to Dr. L. V. Domm through the courtesy of Merck and Company, Inc., was employed in these experiments. The time of pregnancy was determined by observing mating of the rats, or by leaving males with females for a cohabitation period no longer than six hours. The control animals received injections of sterile distilled water in daily doses corresponding to the volume of cortisone administered to the experimental animals. Injections were given subcutaneously. Variation in treatment included dosage (2.50 - 6.50 mg. daily) and length of treatment (7 - 21 days). Daily weight records were kept during period of treatment.

The animals were sacrificed by terminal ether anesthesia on the 18th day of pregnancy or immediately after delivery. Fetuses recovered on the 18th day of gestation were removed by Caesarean section. The criterion used in determining death of fetuses or newborn was the absence of any response to the pinching of toes, tip of tail, or flank with a fine pair of forceps. Observations were made on the effects on the weight of mother and fetus, on the influence on litter size and on the viability and fate of fetuses in utero. Weights were taken on some of the ovaries and uteri of cortisone treated and control animals. The reproductive systems of mother and female fetuses were

examined for gross effects. The ovaries and uteri of experimental and control animals were fixed in either Bouin's fixative or a 10% calcium formalin solution for histological study. The ovaries of some of the newborn rats were removed under a binocular dissecting microscope with the aid of two dissecting needles and a fine eye forceps. Care was taken to leave a part of the uterine horn attached to the ovaries in order not to injure them in technical manipulations with the forceps. The tissues were imbedded in paraffin. Sections were cut at 6 and 8 microns.

Hematoxylin and Eosin were employed for tissue staining. The Periodic Acid Schiff Reaction was used for staining of glycogen and intercellular substance (Lillie, 1954, pp. 123-124). Glycogen was determined by a comparison of sections digested with saliva. Feulgen's Reaction was employed for nuclear stain. Biebrich Scarlet-Picro-Aniline Blue Stain was used for staining of tissues and collagen fibers. Van Gieson's Picric Acid and Acid Fuchsin Stain was employed for muscle tissue and collagen fibers. The above procedures are those given in Lillie (1954).

A total of 128 pregnant and 53 non-pregnant rats were employed in our experiments and 762 fetuses and newborn were recovered and utilized in this study. The initial age of all pregnant and non-pregnant rats employed in our experiments was 90 to 115 days.

EXPERIMENTAL RESULTS

1. Fertility

A study of the effects on fertility was made in a series of 7 mature

female rats. Cortisone (3.50 or 5.00 mg. daily) had been administered in 3 rats for a period of 14 days and in 4 rats for 31 days prior to placing the females with males. The injections were continued during the period of cohabitation which lasted 7 days. Only one of the treated rats became pregnant (3.50 mg. for 38 days) and she delivered 5 living and 1 dead fetus, whereas the 2 control animals became pregnant and delivered 12 and 8 living young (Table VI). The remaining 6 treated females became pregnant between the 35th and 37th days after the cortisone injections were stopped.

2. Observations on Weight

The experimental work in which observations on weight were made was carried out with 24 treated and 12 control pregnant rats. Mating time was determined by leaving males with females for a cohabitation period no longer than 6 hours. Injections in daily doses of 2.50 to 6.50 mg. of cortisone were given for the last 14 to 16 days before parturition. The graphs (Figures 1, 2 and 3) show weight changes for periods of treatment during the last two weeks of gestation. In all cases treated rats showed a loss of weight during the first week of treatment followed by a pregnancy weight increase below that of the controls.

3. Gross Effects on the Reproductive System

Observations were made on the reproductive systems of mother and newborn female rats with the aid of a binocular dissecting microscope immediately after parturition. No evidence of any gross effects with the possible

exception of a size increase of some of the ovaries of treated mothers was observed. Table III shows the differences in weight of ovaries between treated and controls at the time of parturition. An increase of 16.5 to 32% was noticed in pregnant animals treated with cortisone in daily doses of 3.50 to 5.00 mg. for periods of 14 to 16 days as compared with controls (Table IV). Observations were also made on weights of ovaries and uteri in a series of cortisone treated non-pregnant rats (Table V). Daily doses ranged from 3.50 to 5.00 mg. for 14 to 21 days. There was a marked increase in ovarian weights (11.4 - 32.3%) whereas weights of uteri did not show any perceptible changes between treated and control animals.

4. Mortality Rate

The mortality rate of young at birth was studied in 3 series of rats receiving cortisone injections during pregnancy. Variation in treatment included dosage (2.50 - 5.00 mg. daily) and length of treatment (last 7 - 16 days), (Table I). The absence of any response to pinching of toes, tip of tail, or flank was the criterion used in determining death of newborn. The highest mortality rate of newborn rats was observed in the animals treated daily with 5.00 mg. of cortisone for the last 16 days (29.4%). The lowest rate was found in animals treated daily with 2.50 mg. for the last 7 days (7.3%). Mortality rate was in general proportional to the dosage and period of treatment. This evidence did not take into consideration any fetuses which may have died in utero and were resorbed prior to parturition.

5. Effects on Litter Size

There was no significant difference in the number of young at parturition between rats treated daily with 2.50 or 3.50 mg. of cortisone and controls. However, when the daily dose was increased to 5.00 mg., a slight reduction in the size of litters proportionate to the length of the period of treatment was observed (Table I). The corpora lutea of pregnancy were counted histologically in both ovaries removed immediately after parturition from 4 pregnant rats treated with 5.00 mg. of cortisone daily and from 2 pregnant rats treated with 6.50 mg. of cortisone daily for the last 2 weeks of gestation. No numerical difference in the number of corpora lutea of pregnancy was found in comparison with the number of newborn of these treated rats.

6. Effects on Weight of Young at Birth

The newborn of treated pregnant rats were weighed at birth and compared with the birth weights of controls (Table VII). No significant differences in weight were found in the young of mothers treated with 2.50 or 3.50 mg. of cortisone daily. Our data indicate a slight decrease in the average body weight of the young born of mothers treated with a daily dosage of 5.00 mg., however, the decrease is not proportional to the period of administration.

7. Effects on Fetuses Delivered on the 18th day of Gestation

This study was carried out on 11 pregnant animals treated with dosages of 3.50 and 5.00 mg. of cortisone daily. Injections were given for a period of ten consecutive days prior to delivery by Caesarean section, under ether

anesthesia, on the 18th day of gestation. At the time of Caesarean section uterine horns were opened as quickly as possible, fetuses removed, and living and dead segregated and examined. In cases where death was not immediately apparent the criterion of response to pinching was employed.

When the effect of treatment on the mortality rate was analysed it was noticed that a relatively lower number of deaths had resulted in the treated recovered by Caesarean section on the 18th day than was observed in normally born young of cortisone treated rats (Tables I and II). This would indicate that at least some of the fetuses of treated rats die between the 18th day of pregnancy and termination of gestation. A noteworthy difference was apparent in the weights of the fetuses of treated and control rats. The latter were 5.2 to 7.3% heavier as shown in Table VIII. The above results lend credence to earlier observations by others of the inhibitory effect of cortisone on development and growth.

8. Histological Effects on Ovaries of Pregnant Rats

The gain in weight of the ovaries of cortisone treated animals (Table III) prompted us to make histological observations. The ovaries of 24 cortisone treated pregnant and of 12 control pregnant rats were studied histologically. Doses varying from 2.50 to 6.50 mg. of cortisone per day were given for the last fourteen days of gestation. The ovaries employed in this experiment were removed immediately after parturition. The histological examination revealed an increase in the size of corpora lutea and a more advanced follicular

development as indicated by the number and size of the antra (Plates IV and V, figures 7 and 8). We also observed an increase in the size and number of blood vessels of treated ovaries and a transformation of stroma cells causing hyperplasia and hypertrophy.

9. Histological Effects on Ovaries of Non-Pregnant Rats

These observations are based on 12 non-pregnant mature female rats treated with either 3.50 or 5.00 mg. of cortisone daily for a period of fourteen days. Four controls were used. A greater degree of follicular development was observed in the treated animals. We not only observed a greater number of follicles but also an increase in the size of the antra of many of the follicles (Plates I and II, figures 4 and 5).

An increase in the size of the corpora lutea was also noticed in the cortisone treated rats (Plates I and III, figures 4 and 6). Treated ovaries also showed a greater degree of vascularisation. In addition, an increase in the amount of follicular glycogen as determined by the PAS method was observed. The stage of the ovarian cycle was not determined at the time the animals were sacrificed.

10. Histological Effects on Uterine Horns

The uterine horns of 12 mature female rats, treated with 5.00 mg. of cortisone daily for a period of 21 days, were studied histologically and compared with the uterine horns of 4 control animals. Sections used in this comparison were taken from the middle portion of each horn. Our study revealed

no perceptible differences in cross sections of uterine horns between treated and control animals.

11. Histological Effects on Ovaries of the Newborn Rat

Since the observations on adult pregnant and non-pregnant rats indicated differences in weight and histological structure of ovaries, resulting from the administration of cortisone, a histological study was conducted with the purpose of evaluating possible effects on the ovaries of newborn rats whose mothers had received injections of cortisone during pregnancy. Twelve newborn female rats taken from various litters in which the mothers had been treated daily with 3.50 mg. of cortisone for the last 7 days of gestation, were used in this experiment. They were compared with 6 newborn females from non-treated control rats. A relatively smaller number of germinal epithelial cords in the central portion of the ovary was found in the ovaries of newborn from cortisone treated mothers (Plates VI, VII, VIII and IX, figures 9, 10, 11 and 12). Further studies will be undertaken to confirm and extend our observations on the effects of cortisone in fetal ovaries when administered during pregnancy.

DISCUSSION

The experiments conducted in this study have produced definite indications that the administration of cortisone has an effect on pregnancy. Our evidence suggests that when cortisone is administered during pregnancy it may effect the fetus adversely. The increased mortality rate of fetuses, the growth retarding effect on the ovary of the fetus, the effect on body weight of fetuses

delivered on the 18th day of gestation, and other effects observed in our experiments, seem to confirm the observation that cortisone is not only injurious to the fetus, when administered to the mother during pregnancy, but support the assumption that cortisone, or some metabolite of cortisone, crosses the placental barrier. This assumption is supported by evidence from the studies of others. According to Jones, Lloyd, and Whatt (1952), the administration of cortisone in pregnant rats caused a rise in the fetal adrenal cholesterol. Fraser and his associates (1952) produced anomalies in fetal mice by administering cortisone for four days at various stages of pregnancy. The question whether cortisone as such, or as one of its metabolites, crossed the placental barrier in the above experiments is still open and seems to be an important objective in studies on placental transfer.

An analysis of the results obtained in our study, on the influence of cortisone on fertility, confirms the conviction that the effect of cortisone is especially great in this aspect. Our results in general concur with those of Novak (1956), whose study was made on a series of 11 mature female rats, given 3.5 and 5.0 mg. of cortisone daily for periods from 22 to 58 days prior to copulation. Not one of the mated cortisone treated rats conceived although copulation was observed to ensue in each case.

The influence of cortisone on fertility may be caused by its suppression of ACTH production or release. The experiments of Sayers and Sayers (1949) showed that the administration of cortisone inhibits the release of ACTH. It is therefore believed that the influence of cortisone on fertility may be caused by

its inhibition of ACTH release and that the consequent cortical hypofunction may be responsible for sexual aberrations including the disturbances in fertility observed in our experiments.

In our experiments pregnant rats receiving daily injections of 3.5 to 6.5 mg. of cortisone for the last 14 days of gestation showed a loss in body weight during the first week of treatment followed by a pregnancy weight increase markedly below that of controls. The loss in weight during the first week of treatment is apparently not due to a decrease in litter size or weight of fetuses, but to a loss of body fluids of the mother.

There has been considerable discussion concerning the problem of weight loss as a consequence of cortisone administration, but it is not yet known whether carbohydrate metabolism or electrolyte and water balance is involved. It was shown by Ingle and Thorn (1941) and others that cortisone and hydroxycorticosterone cause a temporary increase in the excretion of sodium, chloride, and potassium. In the excretion of these substances water is removed from the body thus causing a decrease in body weight. On the other hand, Sprague et ala (1950) found that cortisone in daily doses of 100 mg., for twelve to thirty days in the human, had little or no effect upon the excretion of calcium, sodium, chloride, and potassium. According to the latter workers, there seems to be an unidentified principle in adrenal cortical extracts which affects the metabolism of fat. The question therefore remains whether cortisone causes loss of weight via the pituitary-adrenal relationship or through a direct influence on the removal of body water from the mesenchymal derived tissues.

The pregnancy weight increase observed during the last 7 days of gestation in our experiments on cortisone treated, pregnant rats is apparently due to a gain in body weight of the developing fetuses during this period. This observation is supported when one compares the weights of normal newborn rats with fetuses delivered on the 18th day of gestation by Caesarean section. An increase of 200 to 300% in body weight was observed in the last 3 days of gestation.

There seems to be no doubt that cortisone influences the viability of fetuses in utero when administered to pregnant rats. A number of workers have reported evidence favoring this observation. Thus, Davis and Plotz (1954), showed that daily administration of 3.5 mg. of cortisone to pregnant rats for 16 to 19 days caused a mortality rate of 47.7%. According to De Costa and Abelman (1952) two types of damage result from daily injections of 5.0 to 20.0 mg. of cortisone in the rabbit during pregnancy. These injuries they maintained may be described as concurrent or delayed, viz., "The concurrent effect is manifested by fetal degeneration or abortion. The delayed effect is observed when fetal growth continues after cortisone is stopped, only to result in still birth or neonatal death." The same authors observed that a few injections in late pregnancy produced damage similar to that observed after many injections in early pregnancy. DeCosta and Abelman (1952) maintain that the rabbit becomes increasingly sensitive to the effects of cortisone as pregnancy progresses. Our experimental data in the albino rat do not seem to support this observation. We observed that the mortality rate was in general proportional, not only to

the dosage administered but also to the length of the treatment period. It is not known whether the effect of cortisone on the viability of fetuses in utero is due to a direct effect on the body metabolism, or whether adrenal atrophy, caused by a suppression of the secretion of the adrenocorticotrophic hormone, is the cause of the mortality of fetuses in cortisone treated pregnant rats.

DeCosta and Abelman (1952) reported that the administration of cortisone in moderate dosages in the human did not seem to interfere with fetal development or survival. The clinical reports of Katzenstein and Alston (1954) suggest that steroid therapy during pregnancy does not result in damage to the infant. It should be noted however that the amount of cortisone administered in pregnant animals is generally much higher, on a body weight basis, than that administered to pregnant women. The high dosages used in experimental animals seem to be beyond the physiological and therapeutic level and this may be the principal cause of damage to the fetus. The dosage employed in human therapy (50 - 150 mg. per day) corresponds on a body weight basis to a dosage equivalent to 0.3 to 1.0 mg. per day in the rat. Since evidence does not confirm the assumption that such low dosages of cortisone interfere with the survival of fetuses in the rat, it is reasonable to assume that in the human prolonged administration of cortisone in dosages beyond the therapeutic level would also effect the viability of the fetus. The report of Leroy and Domm (1951), that injections of 500 gamma of cortisone given fetuses in utero, between the 11th and 16th days of gestation, killed all embryos, whereas, when the same dose was administered between the 16th and 19th days embryos survived and were normal in

size, can also be explained on a dosage to weight basis. The dosage employed in fetuses before the 16th day of gestation was not sufficient on a weight basis to cause mortality when injected after the 16th day of gestation. If this is true then it is important to take into consideration the increase in body weight of fetuses which in the last days of gestation in the rat is relatively very high.

A problem which deserves special attention is the effect of cortisone on the weight of the young at birth. In our experiments, no significant difference in weight was observed in the young of mothers treated daily with 2.50 or 3.50 mg. of cortisone. A slight decrease in the average body weight was found in the young of mothers treated with a daily dosage of 5.00 mg., although the decrease was not proportional to the period of treatment. Our data are in agreement with the observations of Domm and Leroy (1951), who observed a slight decrease in the weight of newborn rats only in cases were dosages of 5.00 and 6.25 mg. had been administered to the mother during pregnancy. In our experiments a significant deficit in body weight (5.2 - 7.3%) was found in the fetuses of pregnant rats treated daily with 3.50 and 5.00 mg. of cortisone for a period of 10 consecutive days. This treatment was prior to delivery by Caesarean section on the 18th day of gestation. The slight deficit in body weight of normally born rats of mothers treated with high dosages of cortisone, and the relatively high deficit found in fetuses delivered by Caesarean section on the 18th day of gestation favors the view that cortisone administered during pregnancy inhibits growth and development of the fetal albino rat. This result

agrees with the report of Tuchmann-Duplessis and Mercier-Parot (1954) who observed that a dosage of 20 mg. of cortisone administered daily to pregnant rats between the 6th and 16th day of gestation caused a decrease of 10% in the average body weight of the young at birth.

A slight reduction in the size of litters proportional to the length of treatment was observed in our experiments in pregnant rats treated daily with 5.00 mg. of cortisone. Davis and Plotz (1954) did not find a significant difference between the number of young born of cortisone treated and non-treated rats. However, Courrier and Cologne (1951) reported the resorption and maceration of fetuses in rabbits treated daily for 4 to 7 days with 2.5 mg. of cortisone. Velardo (1957) observed an effect of ACTH on litter size in albino rats. Female rats were injected daily with 2.0 to 4.0 mg. of ACTH for 5 days prior to cohabitation. All ACTH treated groups produced stillbirths and 3 to 6 rats (16 - 40%) aborted in each group, while none aborted or had stillbirths among the controls. Although no abortions were noticed in our experiments, the slight reduction in litter size found in rats treated with 5.00 mg. of cortisone does not exclude the possibility of resorption in utero. That the latter may have occurred is supported by the fact that a nodule suspected of being the remnant of a macerated fetus was found in a uterine horn among the fetuses of a rat treated with 5.00 mg. of cortisone for 10 days prior to delivery by Caesarean section on the 18th day of gestation.

An increase in the weight of some of the ovaries was observed in our experiments on pregnant cortisone treated rats, when examined immediately after

parturition, as well as in non-pregnant treated rats. This result is in agreement with the observations of Moore (1953) who found the ovaries of cortisone treated rats to be consistently heavier than normals and with those of Blivaiss et ala (1954) who also observed that the ovaries of cortisone treated pair-fed rats were consistently heavier than those of the controls. The observation of Moore (1953), that the fresh weights of uteri did not vary perceptibly between cortisone treated and untreated females, agrees with our findings.

Our histological observations on the ovaries of treated, non-pregnant, mature rats, and on the ovaries of treated, pregnant, rats removed immediately after parturition, revealed a greater degree of follicular development. These ovaries showed a larger number of follicles with antra as well as larger antra and corpora lutea. This finding suggests that the significant weight increase in the ovaries of mature non-pregnant and pregnant cortisone treated rats is due to the increase in size of antra and corpora lutea. This increase could be due to an increased sensitivity of the ovary to gonadotrophic hormone stimulation or to a greater production of gonadotrophins under the influence of cortisone.

In our study on the histology of the ovaries of newborn rats, whose mothers had been treated during the last 7 days of gestation with 3.50 mg. of cortisone per day, we came to the conclusion that these organs had been affected by cortisone. The relatively smaller number of germinal epithelial cords in the central portion of these ovaries may indicate a growth inhibiting effect. The possibility that cortisone inhibits the beta cells of the hypophysis, which

are responsible for the production of gonadotrophic hormones, or that the effect is caused by a reduced sensitivity of the ovaries to gonadotrophic hormones should not be overlooked.

Cortisone when administered in high dosages appears to be deleterious not only when administered during pregnancy but it also seems to interfere with bringing about pregnancy itself. The question whether the administration of cortisone has any effect on ovulation, and if so, through what mechanism the effect is brought about is far from being completely understood. There are contradictory opinions with respect to the physiological manifestations of the process of ovulation. Smith and Ketteringham (1938) determined that the follicular contents of the rat stain very strongly with Best's Carmine, and from this they concluded a glycogen content. According to Deane (1952), glycogen is present in the cumulus oophorus of the follicle of the rat. The rise in the sugar concentration is said to bring about a considerable osmotic absorption of water by the follicle, with the consequent formation of excessive pressure, which may play a role in ovulation (Petry, 1950). Burr and Davies (1951)believe that the liquor represents a transudate from the blood which is brought about by an increased permeability of the vessel walls. The follicles take up the transudate and increase in size to the extent that the liquor accumulates in them. As a result of clamping off the veins, and the consequent rise in pressure, an especially rapid and striking enlargement of the follicles was observed by these investigators.

Several factors are apparently involved in the process of ovulation

although the mechanism causing it has not yet been adequately explained. An increase in pressure inside the follicle is undoubtedly of great significance. However, according to Petry's (1949) investigations, the mechanical factor is not alone in a position to cause the follicle to break but proteinases also play a role by breaking down the collagenous fibers of the follicular wall in the area of the stigma folliculi, and bringing about changes in the connective tissue structure, combined with a decrease in tensile strength. As a result, a small rise in pressure in the interior of the follicle is enough to burst it.

In view of the above considerations and the fact that cortisone increased the vascularization and the glycogen content of follicles in our experiments it seems probable that ovulation is affected by cortisone. Chin-Ye-Chang and Witschi (1957) reported that cortisone was capable of playing a supporting role in the hormonal control of ovulation in the frog. According to these investigators cortisone is effective only if it acts on follicles that have been brought close to the ovulation threshold by other hormonal factors.

The factors controlling corpus luteum formation have not been fully explained, though it is assumed that a certain quantitative balance between the follicle stimulating (FSH) and luteinising (LH) hormones of the anterior lobe of the pituitary is involved. In view of the effect on the development of the follicle and the corpus luteum, observed in our study in cortisone treated rats, it is questionable whether the balance between these two hormones of the anterior lobe of the hypophysis was not disturbed.

According to Weichert and Schurgast (1942) the corpora lutea of preg-

nancy in the rat remain constant at about 1.45 mm. during the first 10 or 11 days when they undergo a rapid increase in size and remain constant at about 2.0 mm. from the sixteenth day until parturition. It has always been puzzling why the corpus luteum persists in pregnancy, but since it does, the assumption of a special hypophyseal effector, luteotropin, as the substance responsible for the maintenance of the corpus luteum has become an accepted view. The fact that the corpus luteum of pregnancy in the albino rat persists and increases in size through the administration of cortisone is additional confirmation of the view that cortisone has a definite effect on pregnancy.

SUMMARY

1. Daily administration of cortisone in dosages of 3.50 to 5.00 mg. for 14 to 31 days prior to cohabitation brought about a reduction in the number of pregnancies.
2. A reduction in normal body weight was observed during the second week and an increase, though noticeably below normal, during the last week of gestation, in animals treated daily with 3.50 to 6.50 mg. of cortisone.
3. The weights of ovaries were significantly greater in cortisone treated animals, whereas the weights of uteri did not vary perceptibly between treated and control animals.
4. The mortality rate of fetuses in utero was in general proportional to the dosage and period of treatment.

5. A slight reduction in litter size proportional to the period of treatment during pregnancy was observed where the daily dosage of cortisone was increased to 5.00 mg.
6. A decrease in the body weight of newborn from mothers treated daily with 5.00 mg. of cortisone was observed, however, the decrease was not proportional to the period of administration.
7. A noticeable decrease in body weight of fetuses delivered by Caesarean section on the 18th day of pregnancy was apparent in mothers treated with 3.50 and 5.00 mg. of cortisone for ten days prior to recovery.
8. The histological examination of ovaries from cortisone treated adult rats revealed an increase in the size of corpora lutea and a greater degree of follicular development.
9. An increase in the vascularization of the ovary, transformations of stroma cells and an increase in the glycogen content of follicles were observed in the ovaries of cortisone treated rats.
10. No perceptible histological effects were evident in cross sections taken from the middle portion of uterine horns in mature, non-pregnant female rats treated daily with 5.00 mg. of cortisone for a period of 21 days.
11. A reduction in the number of germinal epithelial cords was observed in the central portion of the ovary of fetuses from mothers treated daily with 3.50 mg. of cortisone for the last 7 days of gestation.

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TABLE I

MORTALITY RATES OF YOUNG AT BIRTH IN A SERIES

OF PREGNANT CORTISONE TREATED RATS

Period inj.*	Inj. per day mg.	No. of litters	Total No. born	Aver. per litter	% born alive	% born dead
7	2.50	4	41	10.1	92.7	7.3
7	3.50	5	48	9.6	91.7	8.3
7	5.00	4	39	9.7	92.3	7.7
14	2.50	6	61	10.2	86.9	13.1
14	3.50	8	84	10.5	84.7	15.3
14	5.00	10	98	9.8	79.6	20.4
16	2.50	4	39	9.7	87.5	12.5
16	3.50	7	69	9.9	65.4	24.6
16	5.00	9	78	8.6	70.6	29.4
Controls**						
7		8	81	10.1	96.3	3.7
14		7	69	9.9	95.6	4.4
16		6	64	10.6	96.9	3.1

* Days prior to parturition.

** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE II

MORTALITY RATES OF FETUSES RECOVERED BY CAESAREAN SECTION ON THE 18TH DAY OF GESTATION

Period inj.*	Inj. per day mg.	No. of litters	Total No. born	Aver. per litter	% born alive	% born dead
10	3.50	6	65	10.5	86.1	13.9
10	5.00	5	51	10.1	82.4	17.6
Controls**						
10		4	43	10.7	100	0

* Days prior to recovery by Caesarean section.

** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE III

WEIGHTS OF OVARIES IN A SERIES OF NON-PREGNANT CORTISONE TREATED RATS

Rat No.	Inj. per day mg.	No. of injs.**	Total amt. inj. mg.	Weight of ovaries mg. Left	Right	Mean of the aver. wt. mg. *
1	3.50	14	49.00	120	110	
2	3.50	14	49.00	110	125	
3	3.50	14	49.00	120	115	117
4	3.50	14	49.00	115	110	
5	3.50	14	49.00	125	120	P = < 0.001.
6	5.00	14	70.00	125	130	
7	5.00	14	70.00	130	130	
8	5.00	14	70.00	125	130	129
9	5.00	14	70.00	120	135	
10	5.00	14	70.00	130	135	P = < 0.001.
11	5.00	21	105.00	130	125	
12	5.00	21	105.00	160	170	
13	5.00	21	105.00	120	150	
14	5.00	21	105.00	150	125	139
15	5.00	21	105.00	130	150	
16	5.00	21	105.00	140	135	P = < 0.001.
17	5.00	21	105.00	130	135	
Controls***						
1		14	49.00	110	105	
2		14	49.00	100	100	
3		14	70.00	105	100	105
4		14	70.00	100	110	
5		21	105.00	105	110	
6		21	105.00	110	105	

* The mean includes the weights of left and right ovaries.
** Injections were given daily.
*** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE IV

WEIGHTS OF OVARIES IN A SERIES OF PREGNANT CORTISONE TREATED RATS

Rat No.	Inj. per day mg.	No. of inj.**	Total amt. inj. mg.	Weight of ovaries mg.✓ Left	Right	Mean of the aver. wt. mg. *
1	3.50	14	49.00	115	120	
2	3.50	14	49.00	110	115	
3	3.50	14	49.00	135	140	
4	3.50	14	49.00	115	125	120
5	3.50	14	49.00	120	115	
6	3.50	14	49.00	120	115	P = < 0.001.
7	5.00	14	70.00	115	130	
8	5.00	14	70.00	120	150	
9	5.00	14	70.00	130	125	
10	5.00	14	70.00	125	125	131
11	5.00	14	70.00	150	120	
12	5.00	14	70.00	145	130	P = < 0.001.
13	5.00	16	80.00	130	105	
14	5.00	16	80.00	120	140	
15	5.00	16	80.00	140	145	
16	5.00	16	80.00	140	140	136
17	5.00	16	80.00	150	150	
18	5.00	16	80.00	135	145	P = < 0.001.
Controls***						
1		14	49.00	100	105	
2		14	49.00	110	100	
3		14	70.00	95	110	103
4		14	70.00	105	110	
5		16	80.00	105	105	
6		16	80.00	105	105	

* The mean includes the weights of left and right ovaries.
✓ Ovaries were weighed and fixed immediately following parturition.
** Injections were given daily.
*** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE V

WEIGHTS OF UTERI IN A SERIES OF NON-PREGNANT CORTISONE TREATED RATS

Rat No.	Inj. per day mg.	No. of injs.**	Total amt. inj. mg.	Weight of uteri gm.*	Mean of the aver. wt. gm.
1	3.50	14	49.00	1.10	
2	3.50	14	49.00	0.95	
3	3.50	14	49.00	1.15	1.07
4	3.50	14	49.00	1.10	
5	3.50	14	49.00	1.05	P = > 0.05.
6	5.00	14	70.00	1.25	
7	5.00	14	70.00	1.00	
8	5.00	14	70.00	1.15	1.10
9	5.00	14	70.00	1.25	
10	5.00	14	70.00	0.95	P = > 0.05.
11	5.00	21	105.00	1.00	
12	5.00	21	105.00	0.90	
13	5.00	21	105.00	1.10	
14	5.00	21	105.00	1.05	1.03
15	5.00	21	105.00	1.00	
16	5.00	21	105.00	1.15	P = > 0.05.
17	5.00	21	105.00	1.00	
Controls***					
1		14	49.00	1.15	
2		14	49.00	0.95	
3		14	70.00	0.90	
4		14	70.00	1.15	1.06
5		21	105.00	1.10	
6		21	105.00	1.10	

* Uteri were weighed and fixed immediately following parturition.
** Injections were given daily.
*** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE VI

EFFECTS OF DAILY ADMINISTRATION OF

CORTISONE ON FERTILITY

Rat No.	Inj. per day mg.	No. of injs.	Total amt. inj. mg.	Days of cohabitation	No. of young born alive	dead
1*	3.5	38	133.0	7	0	0
2*	3.5	38	133.0	7	5	1
3**	3.5	21	73.5	7	0	0
4*	5.0	38	190.0	7	0	0
5*	5.0	38	190.0	7	0	0
6**	5.0	21	105.0	7	0	0
7**	5.0	21	105.0	7	0	0
Controls***						
1*		38	133.0	7	12	0
2**		21	105.0	7	8	0

* Injections began 31 days before cohabitation.

** Injections began 14 days before cohabitation.

*** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

TABLE VII

EFFECTS OF DAILY ADMINISTRATION OF CORTISONE DURING PREGNANCY ON THE WEIGHT OF YOUNG AT BIRTH

Rat No.	Inj. per day mg.	No. of injs.	Total amt. inj. mg.	Aver. indiv. wt. gm. *	Mean of the aver. indiv. wt.
1	5.00	7	35.0	4.49	
2	5.00	7	35.0	5.14	4.57
3	5.00	7	35.0	4.08	
4	2.50	14	35.0	5.57	
5	2.50	14	35.0	5.18	4.72
6	2.50	14	35.0	3.41	
7	3.50	14	49.0	5.21	
8	3.50	14	49.0	5.32	4.81
9	3.50	14	49.0	3.97	
10	5.00	14	70.0	5.34	
11	5.00	14	70.0	3.79	4.45
12	5.00	14	70.0	4.23	
13	5.00	16	80.0	4.84	
14	5.00	16	80.0	4.21	4.69
15	5.00	16	80.0	5.02	
Controls**					
1		7	35.0	4.64	
2		14	35.0	4.29	
3		14	49.0	5.62	4.75
4		14	70.0	4.41	
5		16	80.0	4.78	

* Newborn were weighed and fixed immediately following parturition.
** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

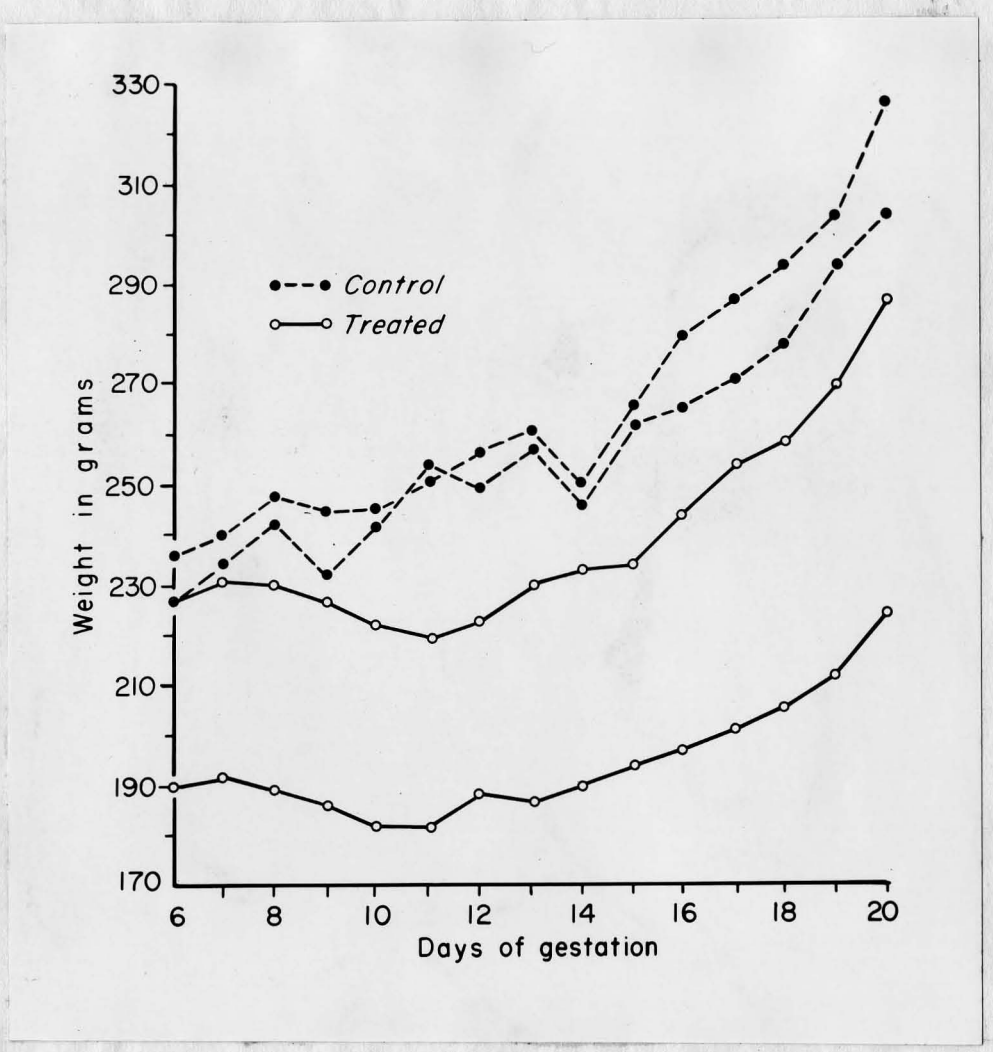
TABLE VIII

EFFECTS OF DAILY ADMINISTRATION OF CORTISONE DURING PREGNANCY ON THE WEIGHT OF FETUSES RECOVERED ON THE 18TH DAY OF GESTATION *

Rat No.	Inj. per day mg.	No. of injs.	Total amt. inj. mg.	Aver. indiv. wt. gm.	Mean of the aver. indiv. wt. gm.
1	3.50	10	35.0	0.96	
2	3.50	10	35.0	0.87	
3	3.50	10	35.0	0.94	
4	3.50	10	35.0	0.99	0.92
5	3.50	10	35.0	0.85	
6	3.50	10	35.0	0.94	
7	5.00	10	50.0	0.95	
8	5.00	10	50.0	0.87	
9	5.00	10	50.0	0.86	0.90
10	5.00	10	50.0	0.90	
11	5.00	10	50.0	0.93	
Controls**					
1		10	35.0	1.04	
2		10	35.0	1.08	1.03
3		10	35.0	0.94	
4		10	35.0	1.07	

* Fetuses were recovered by Caesarean section.
** Injected volume of sterile distilled water equivalent to volume of fluid received by experimentals.

Figure 1. Graph showing the subnormal weight gain observed in pregnant rats that had received daily injections of 3.50 mg. of cortisone during the last 14 days of gestation. (Compare figures 2 and 3) Injections began on the 7th day of gestation.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310
290
270
250
230
210
190
170
Weight in grams
Control
Treated
6
8
10
12
14
16
18
20
Days of gestation

FIGURE 1

Figure 2. Graph showing the subnormal weight gain observed in pregnant rats that had received daily injections of 5.00 mg. of cortisone during the last 14 days of gestation. (Compare figures 1 and 3) Injections began on the 7th day of gestation

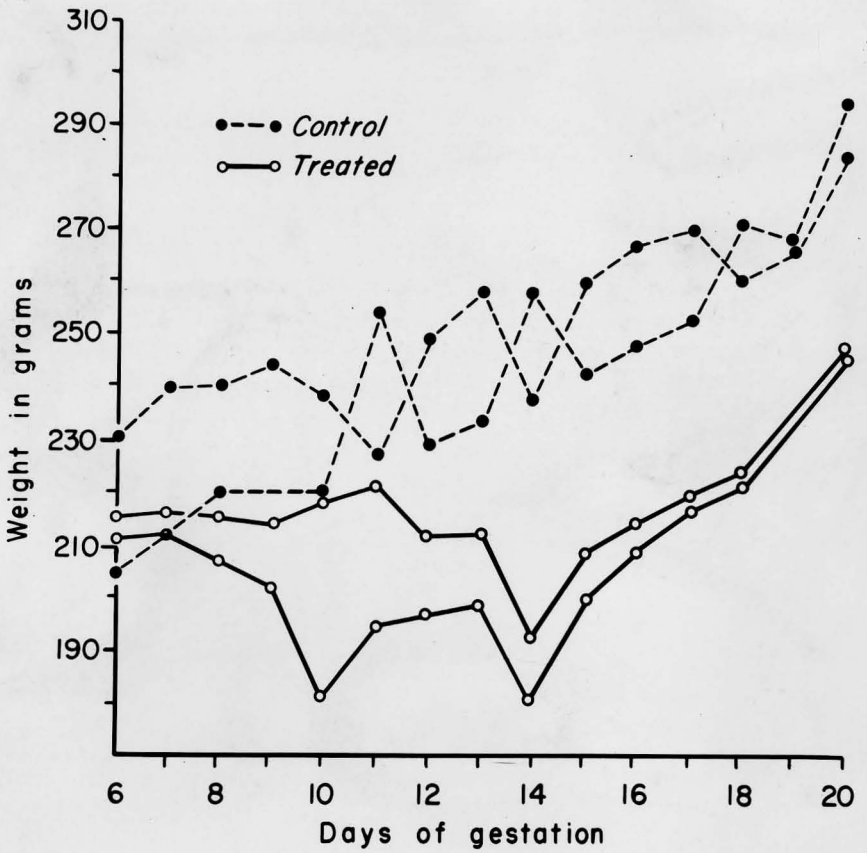

310
290
270
250
230
210
190
Weight in grams
Control
Treated
6
8
10
12
14
16
18
20
Days of gestation

FIGURE 2

Figure 3. Graph showing the subnormal weight gain observed in pregnant rats that had received daily injections of 6.50 mg. of cortisone during the last 14 days of gestation. (Compare figures 1 and 2) Injections began on the 7th day of gestation.

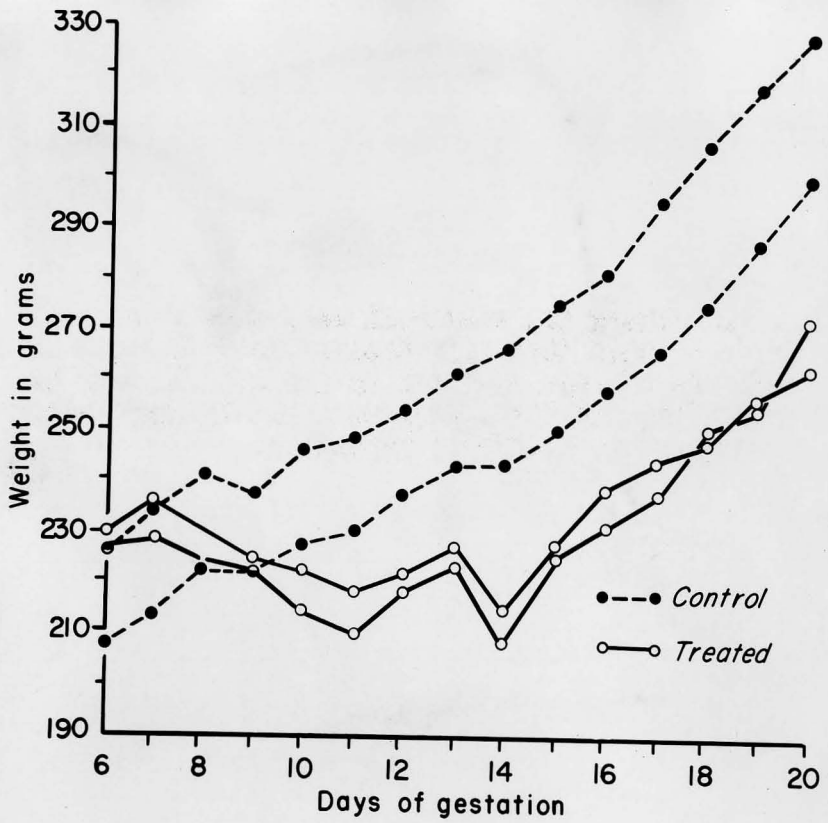
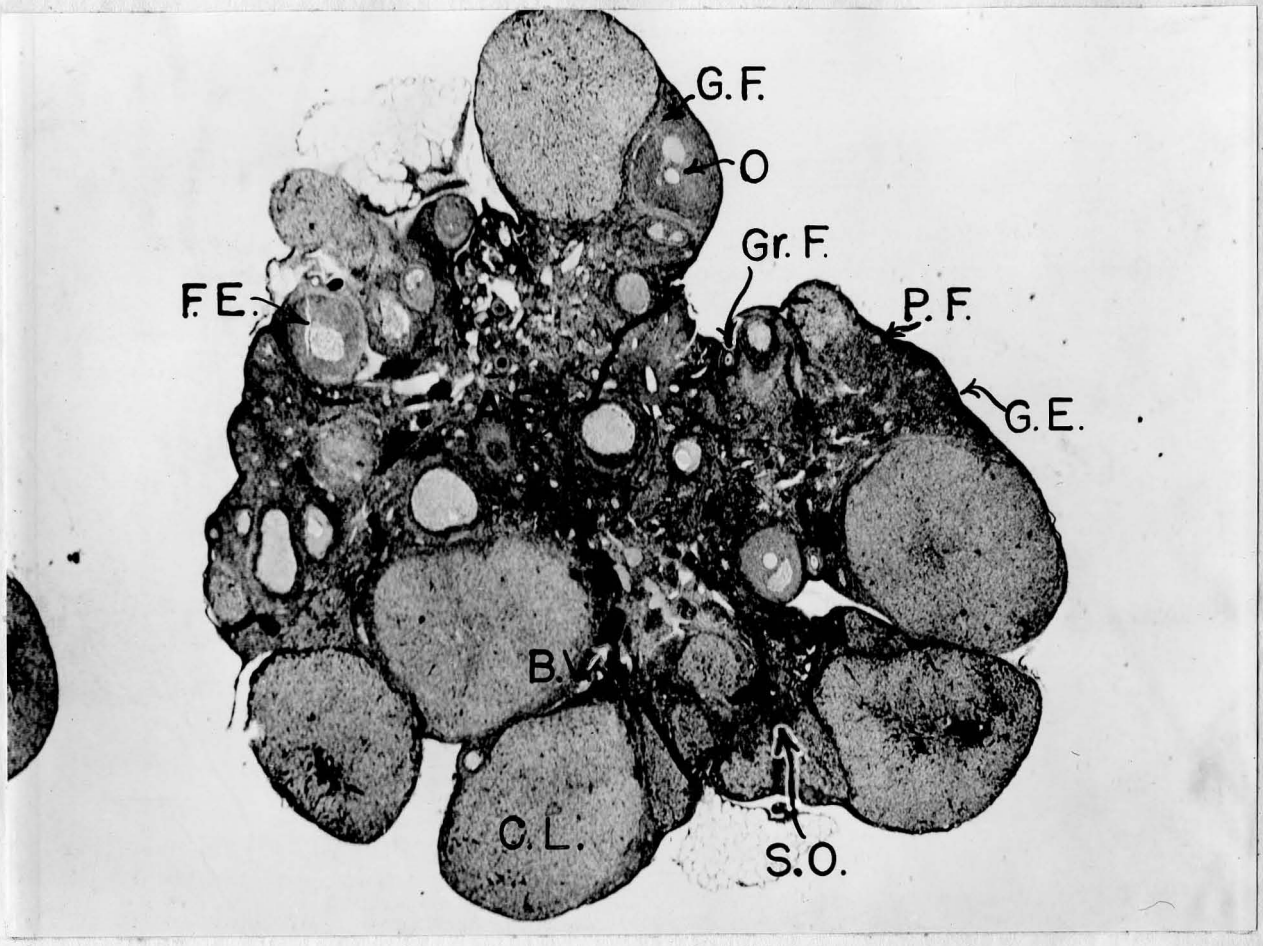

330
310
290
270
250
230
210
190
Weight in grams
6
8
10
12
14
16
18
20
Days of gestation
Control
Treated

FIGURE 3

PLATE I

Figure 4. Ovary from a control non-pregnant rat, age 97 days. Material was fixed in Bouin's fixative and stained with Hematoxylin and Eosin. X 40.

S.O. - Stroma ovarii
G.E. - Germinal epithelium
P.F. - Primordial follicle
Gr.F.- Growing follicle
F.E. - Follicular epithelium
O. - Ovum
G.F. - Graafian follicle
B.V. - Blood vessel
C.L. - Corpus luteum
A.F. - Atretic follicle



FIOURE 4

PLATE II

Figure 5. Ovary from a non-pregnant rat, 97 days old, treated daily with 3.50 mg. of cortisone for 14 days. Note the great number of follicles and the increased size of the antra. (Compare figure 4, plate I) Ovary was fixed in Bouin's fixative and stained with Hematoxylin and Eosin. X 40.

G.F. - Graafian follicle
F.E. - Follicular epithelium
C.O. - Cumulus oophorus
Gr.F. - Growing follicle
C.L. - Corpus luteum
S.O. - Stroma ovarii
OV. - Oviduct

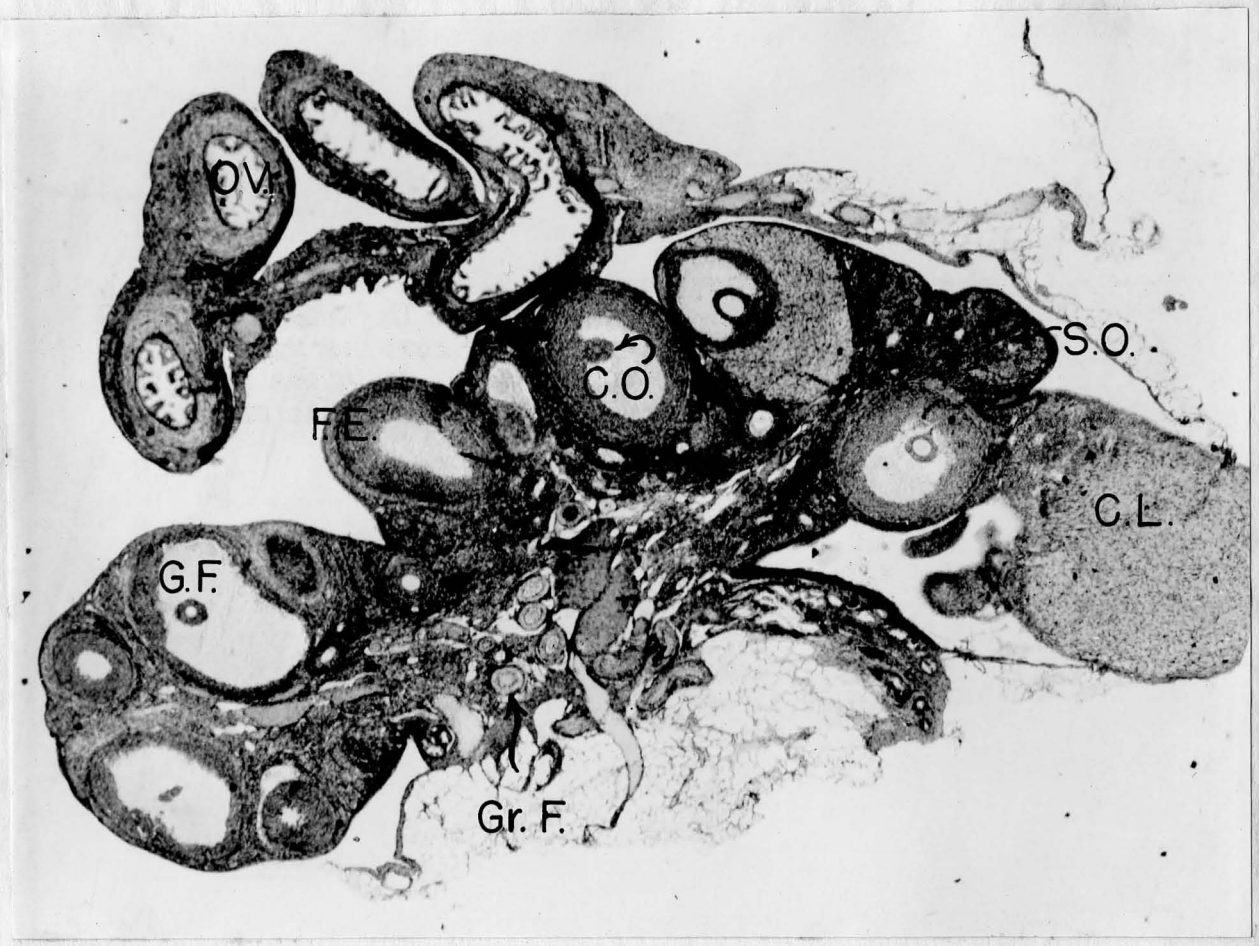


FIGURE 5

PLATE III

Figure 6. Ovary from a non-pregnant rat, 97 days old, treated daily with 6.50 mg. of cortisone for 14 days. Note the large corpora lutea. (Compare figure 4, plate I and figure 5, plate II) Material was fixed in Bouin's fixative and stained with Hematoxylin and Eosin. X 40.

G.F. - Graafian follicle
F.E. - Follicular epithelium
O. - Ovum
C.L. - Corpus luteum
OV. - Oviduct
P.F. - Primordial follicle
C.A. - Corpus albicans
A.F. - Atretic follicle

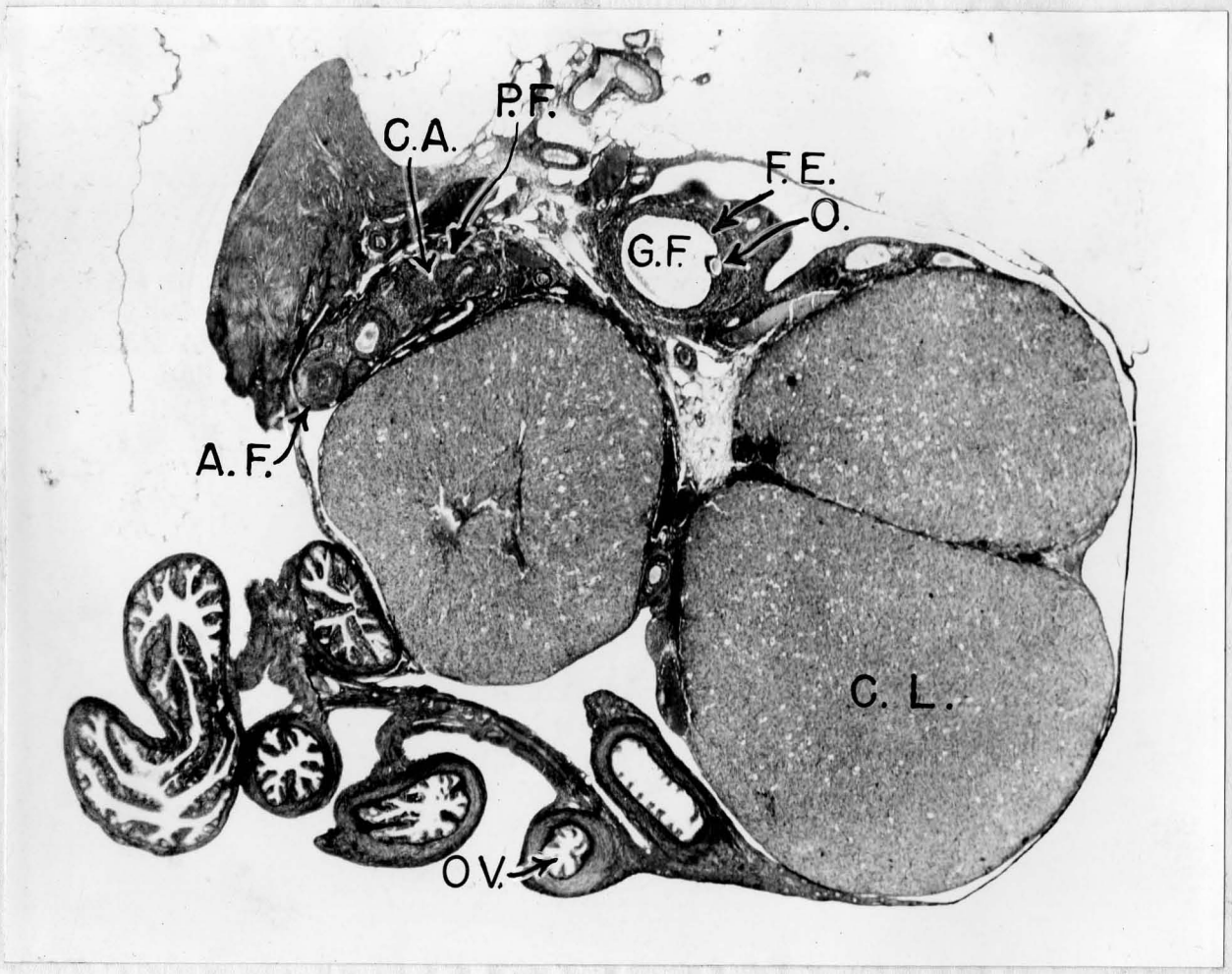


FIGURE 6

PLATE IV

Figure 7. Ovary removed immediately after parturition from a control pregnant rat, age 124 days. Material was fixed in Bouin's fixative and stained with Hematoxylin and Eosin. X 40.

G.F. - Graafian follicle
S.O. - Stroma ovarii
B.V. - Blood vessel
Gr.F.- Growing follicle
A.F. - Atretic follicle
C.L. - Corpus luteum
OV. - Oviduct
F.E. - Follicular epithelium
O. - Ovum

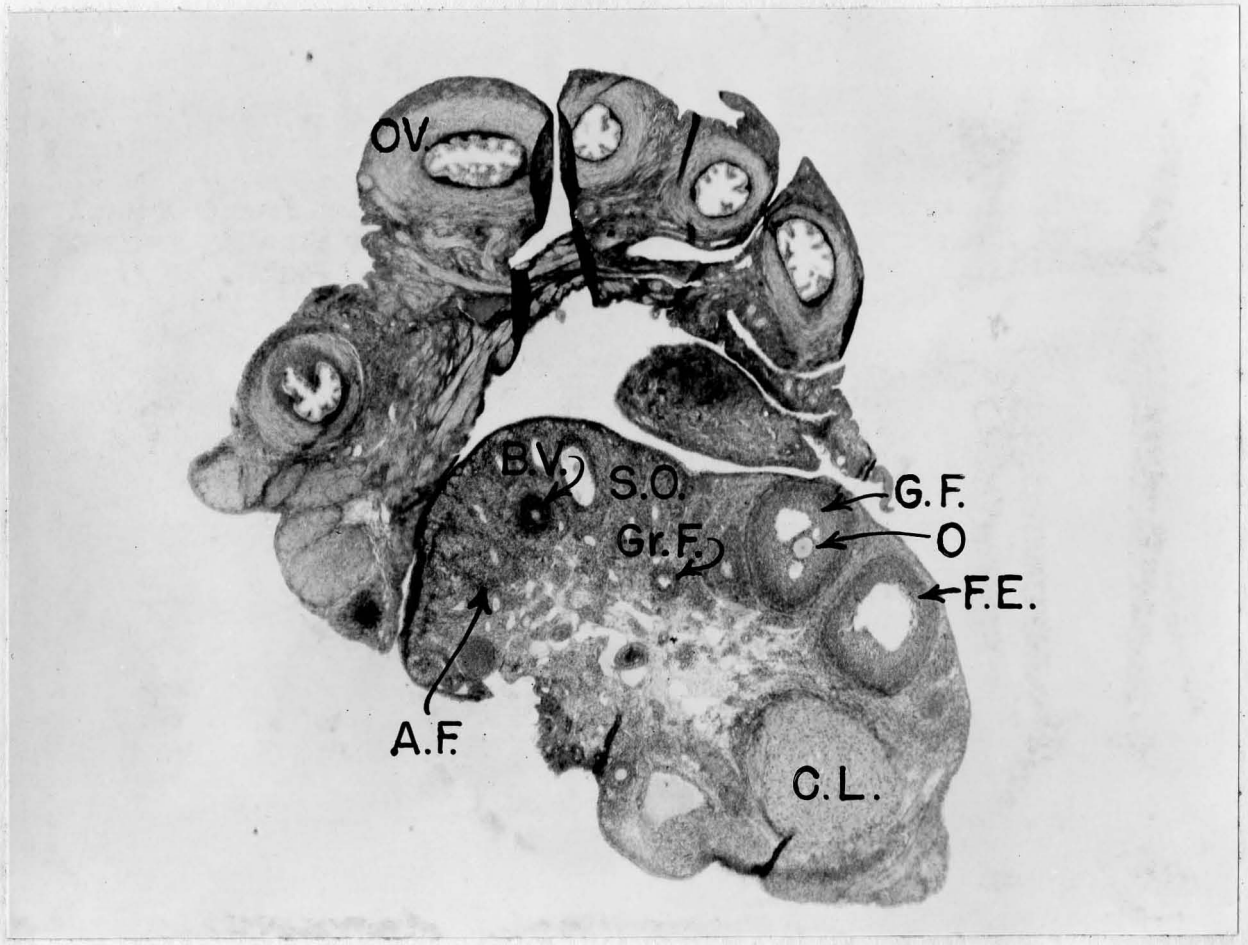


FIGURE 7

PLATE V

Figure 8. Ovary removed immediately after parturition from a 12½ day old pregnant rat treated daily with 5.00 mg. of cortisone for the last 14 days of gestation. Note the large corpora lutea of pregnancy. (Compare figure 7, Plate IV) Material was fixed in Bouin's fixative and stained with Hematoxylin and Eosin. X 40.

G.E. - Germinal epithelium
B.V. - Blood vessel
A.F. - Atretic follicle
Gr.F.- Growing follicle
S.O. - Stroma ovarii
P.F. - Primordial follicle
F.E. - Follicular epithelium
C.L.P.- Corpus luteum of pregnancy

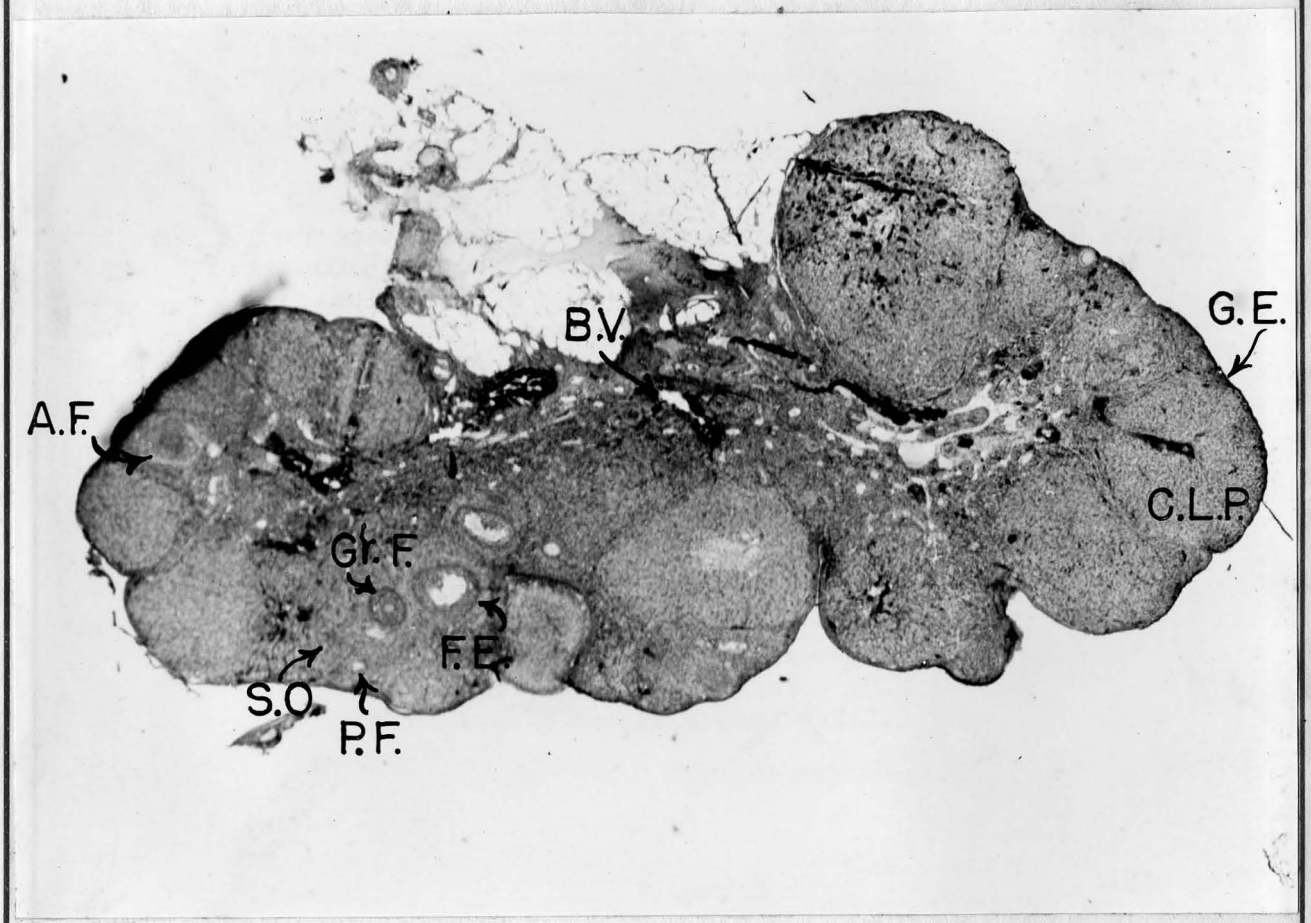


FIGURE 8

PLATE VI

Figure 9. Ovary from a control newborn rat sacrificed immediately after parturition. Material was fixed in Bouin's fixative and stained with Biebrich Scarlet-Picro-Aniline Blue. X 150.

P.C. - Primary cord
S.C. - Secondary cord
N. - Nucleus
C. - Cytoplasm
M. - Medulla

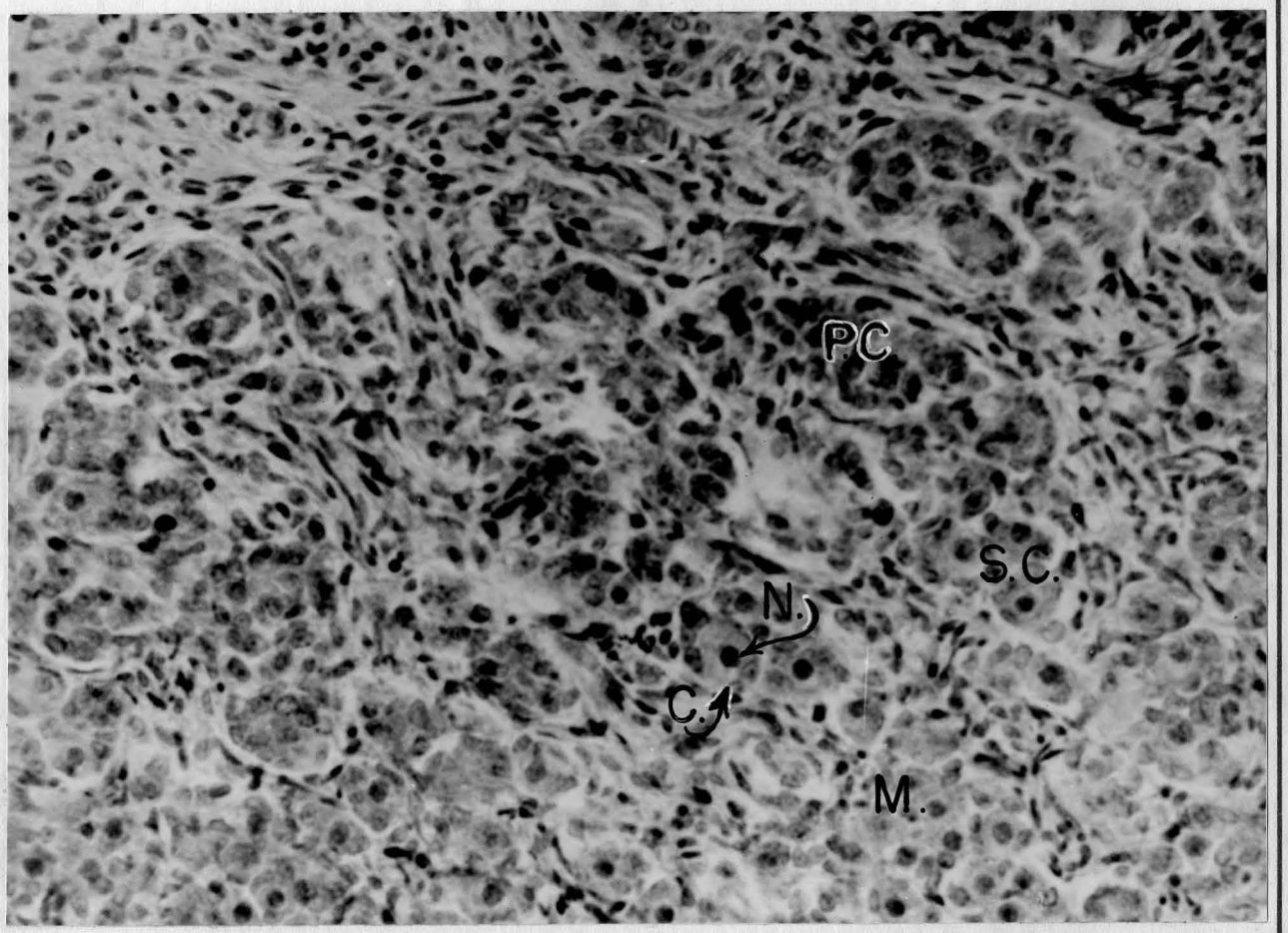


FIGURE 9

PLATE VII

Figure 10. Ovary from a newborn rat, sacrificed immediately after birth, whose mother had received daily injections of 3.50 mg. of cortisone for the last 7 days of gestation. Note the small number of germinal epithelial cords in the central portion of the ovary. (Compare figure 9, plate VI) Material was fixed in Bouin's fixative and stained with Biebrich Scarlet-Picro-Aniline Blue. X 150.

G.E. - Germinal epithelium
P.C. - Primary cord
N. - Nucleus
C. - Cytoplasm
M. - Medulla

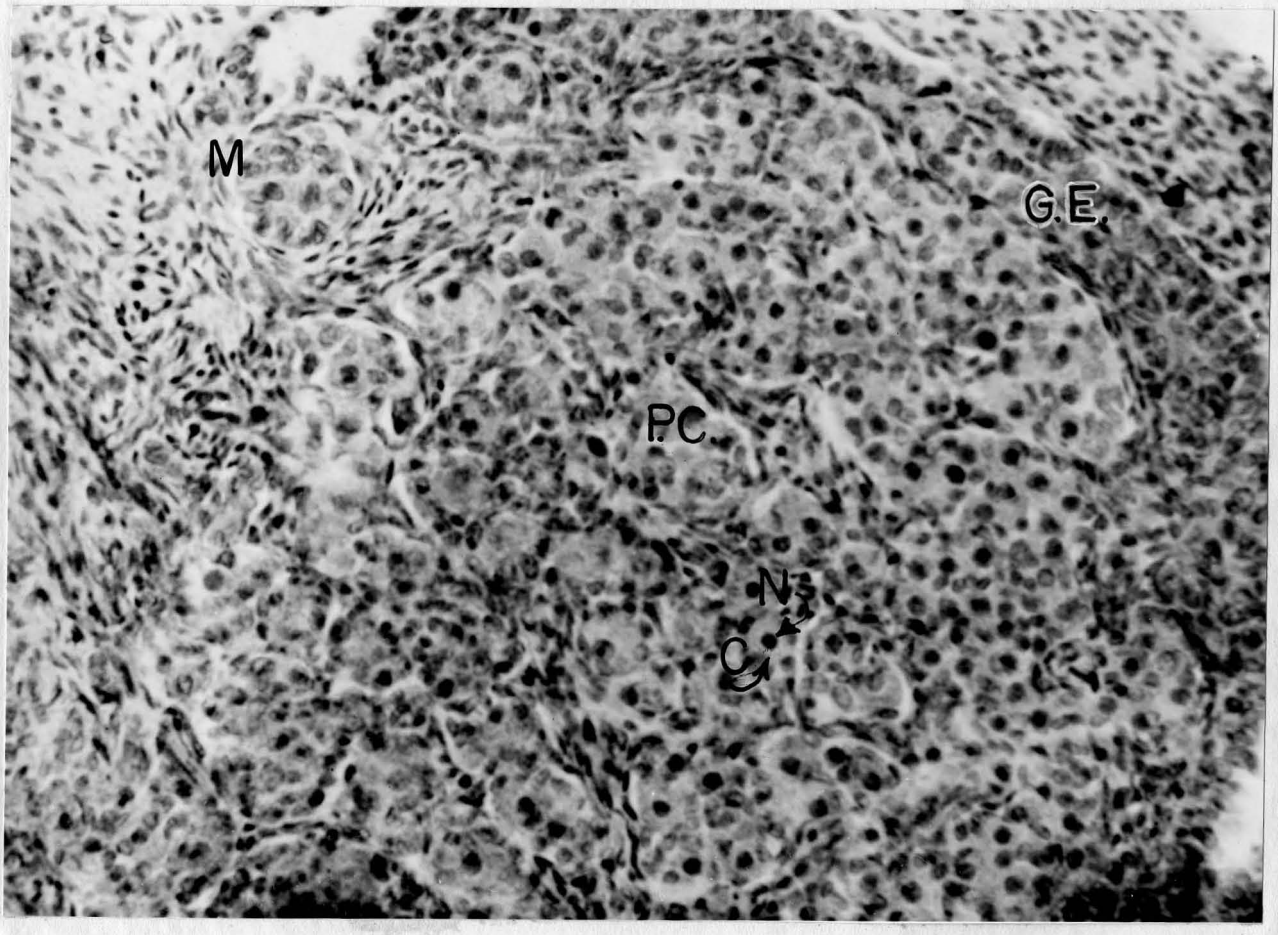


FIGURE 10

PLATE VIII

Figure 11. Higher magnification of a portion of the ovary from control newborn rat shown in figure 9, plate VI. Material was fixed in Bouin's fixative and stained with Biebrich Scarlet-Picro-Aniline Blue. X 1000.

P.C. - Primary cord
S.C. - Secondary cord
O. - Oogonia
N. - Nucleus
M. - Medulla

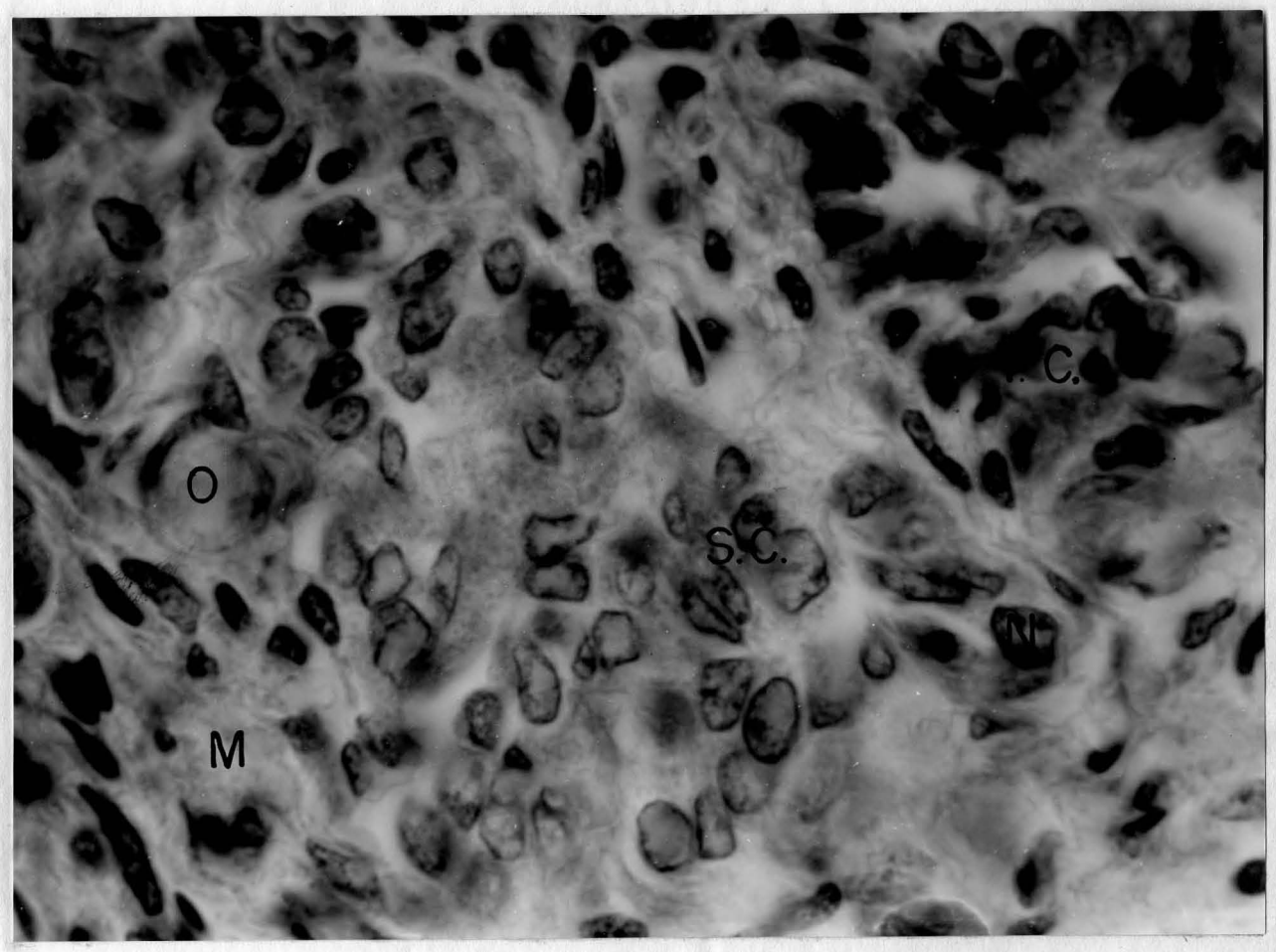


FIGURE 11

PLATE IX

Figure 12. Higher magnification of a portion of the ovary from newborn rat shown in figure 10, plate VII. The mother had received daily injections of 3.50 mg. of cortisone for the last 7 days of gestation. Material was fixed in Bouin's fixative and stained with Biebrich Scarlet-Picro-Aniline Blue. X 1000.

P.C. - Primary cord
O. - Oogonia
N. - Nucleus
C. - Cytoplasm

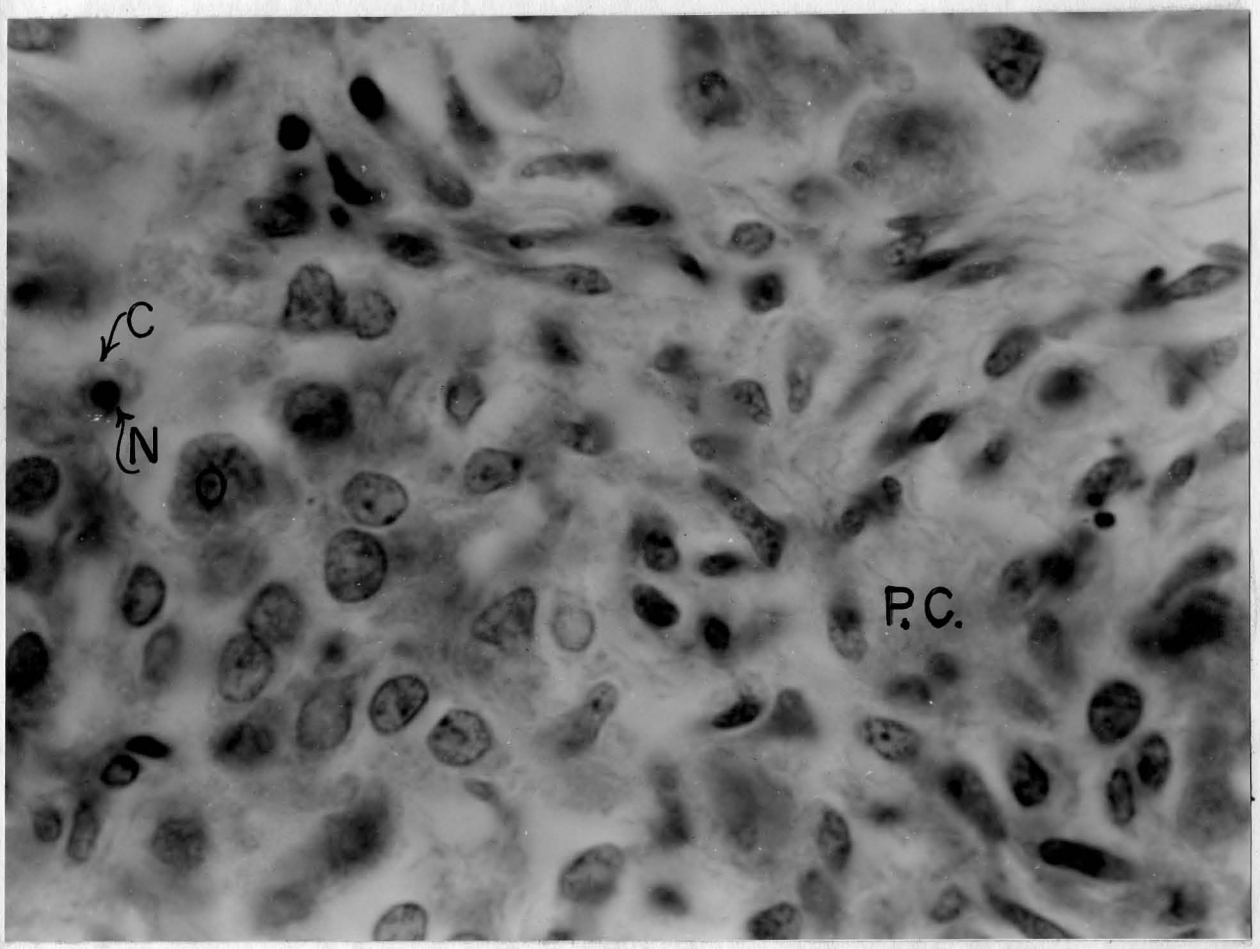


FIGURE 12

APPROVAL SHEET

The thesis submitted by Samuel Perelmuter has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

6/4/58
Date

Signature of Adviser